Use of Microwave Assited Extraction to obtain anthocyanins and total phenolic compounds rich extracts in myrtle (*Myrtus communis L.*)

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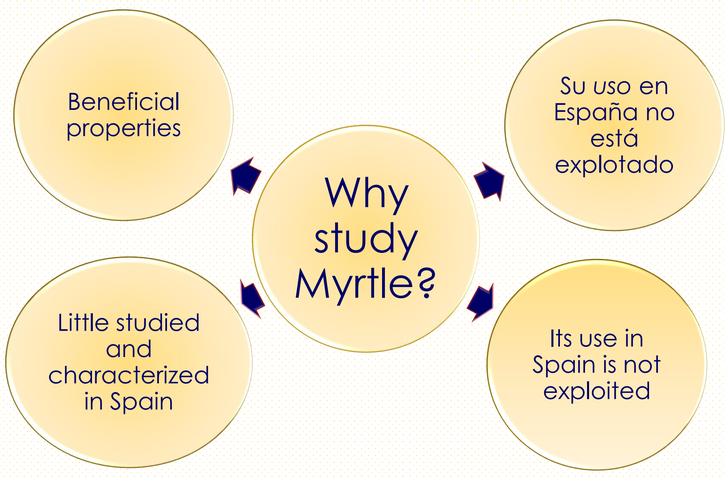
INTRODUCTION

- Myrtle is the fruit of the Myrtle shrub (*Myrtus communis* L.).[1]
- It belongs to the genus *Myrtus* and the family *Myrtacea*.
- It grows spontaneously in the Mediterranean area and the Middle East.[2]
- This species is a very aromatic plant because of the high essential oil content in its leaf, flower and fruit glands.[3]
- It contains a huge concentration of antioxidant substances (anthocyanins and phenolic compounds), much higher than other fruits.[4]
- It is used to produce the charasteristic myrtle liqueur typical of Sardinia.[5]
- Anthocyanins and phenolics have potential health-promoting effects (i.e., antioxidant, anti-inflammatory, and anticancer activities).[6]



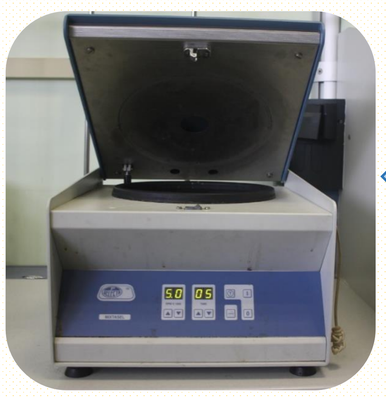
MATERIALS AND METHODS

MICROWAVE ASSISTED EXTRACTION

❖ 0.5 grams

❖ Extraction time: 5 min

❖ 7500 rpm; 5 min



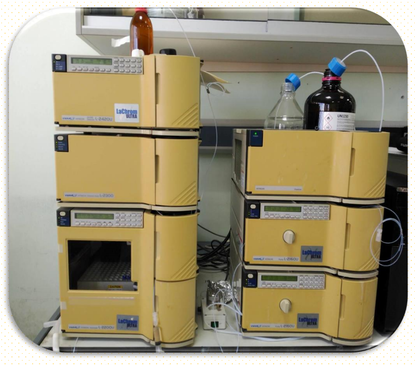
❖ Keep at -20 ºC until analysis

❖ Microwave MARS 6 240/50 (one touch technology, CEM Corporation Matthews, North Carolina, United State).
❖ Power: 800 W.

QUANTIFICATION OF PHENOLIC COMPOUNDS BY THE FOLIN-CIOCALTEAU METHOD

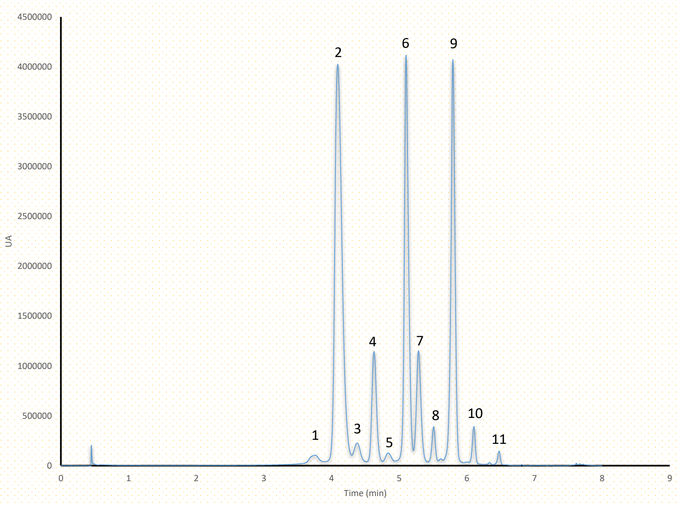
Spectrophotometer UV-Vis Helios gamma (γ) (750 nm)

SEPARATION, QUANTIFICATION AND IDENTIFICATION OF ANTHOCYANINS



HPLC Elite LaChrom Ultra System
Column "Fused Core" C-18
Volumn of injection: 15 µL
Solvent A: Water (5% formic acid)
Solvent B: Methanol
Flow: 0.7 mL/min
Temperature of the column: 50 ºC
Analysis time less than 8 minutes

Time (min)	0.0	1.5	3.3	4.8	5.4
% solvent. B	0	5	15	25	40



1. Delphinidin 3,5-*O*-diglucoside; 2. Delphinidin 3-*O*-glucoside; 3. Cyanidin 3-*O*-*galactoside; 4. C*yanidin 3-*O*-glucoside; 5. Cyanidin 3-*O*-arabinoside; 6. Petunidin 3-*O*-glucoside; 7. Delphinidin 3-*O*-arabinoside; 8. Peonidin 3-*O*-glucoside; 9. Malvidin 3-*O*-glucoside; 10. Petunidin-3-O-arabinoside; 11. Malvidin-3-O-arabinoside.

RESULTS AND DISCUSSION

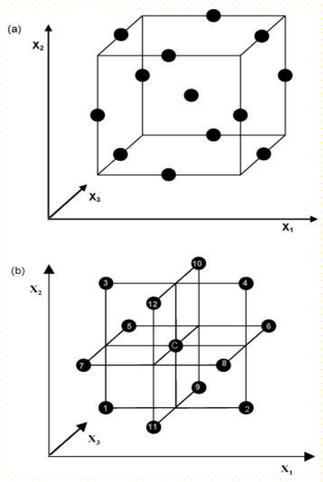
EXPERIMENTAL DESIGN (BOX BEHNKEN)

27 duplicate extractions (1 block)
4 Factors (3 Levels):

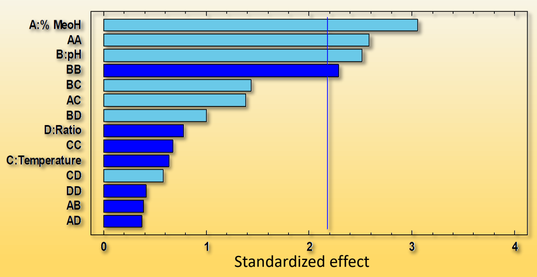
%MeOH	pH	Temperature (ºC)	Ratio (mL)
25 – 50 – 75	2 – 4,5 – 7	10 - 35-60	10 – 15 – 20

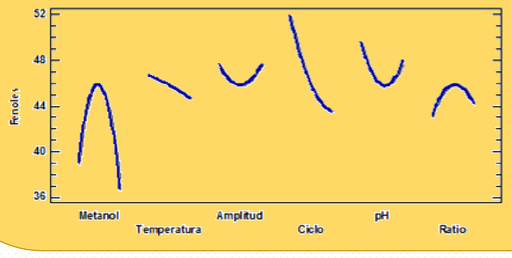
2 Responses: Total anthocyanins and total phenolics

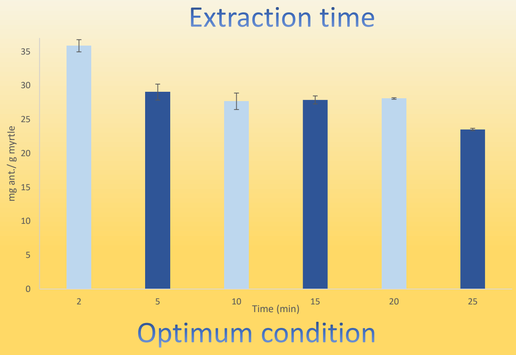
Statgraphics Centurion Version XVII



TOTAL PHENOLICS



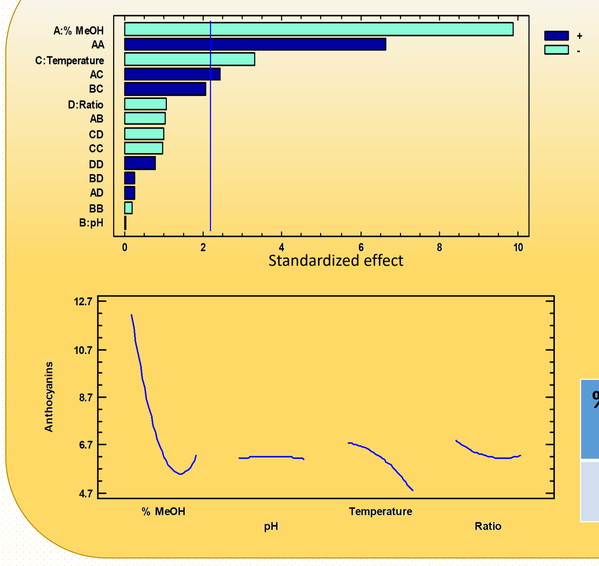


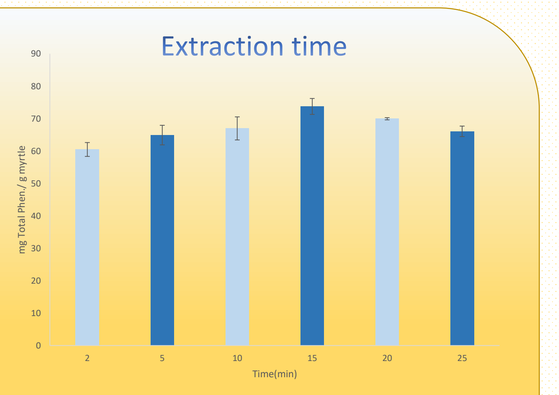


Optimum condition

% MeoH in water	pH	Temperature (ºC)	Ratio	Time (5 min)
59.64	2	100	20	15

ANTHOCYANINS





Optimum condition

% MeoH in water	pH	Temperature (ºC)	Ratio	Time (5 min)
50	3,33	50	20	2

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CONCLUSIONS

- **Two methods for the extraction of total anthocyanins and total phenolic compounds in myrtle by Microwave Assited Extraction have been developed.**
- **A Box-Behnken experimental design with 4 factors and 2 responses (one for anthocyanins and one for phenolic compounds) has been carried out.**
- **HPLC has been used for the identification and analysis of the anthocyanins, and Folin Cioacalteu method was used for the analysis of total phenolic compounds.**
- **According to the experimental design, the percentage of methanol in the extraction solvent, the interaction percentage of methanol in the extraction solvent-percentage of methanol in the extraction solvent and the pH are the determining factors for the extraction of total phenolic compounds.**
- **According to the experimental design, the interaction percentage of methanol in the extraction solvent-percentage of methanol in the extraction solvent, temperatura and the interaction pH-temperature are the determining factors for the extraction of anthocyanins.**
- **Different extraction times were tested using the optimal conditions of extraction. A extraction time of 15 minutes was enough for quantitative extraction of total phenolic compounds and a extraction time of 2 minutes was enough for anthocyanins.**
- **The developed methods presented a high repeatability and reproducibility (DER < 5%).**